Entered
1/31/08

Final Narrative Report

PI: David I. Shreiber
Graduate Fellow: Jason Maikos
Institution: Rutgers, the State University of New Jersey
Grant Title: In vivo thresholds for spinal cord injury
Grant #: 04-2903-SCR-E-0
Grant Period Covered: 6/15/04 – 6/30/07
Date of Submission: 9/26/07

1. Specific Aims

The broad, long term objective of this research is to identify the conditions that cause spinal cord injury (SCI) in humans. The goal of this proposal is to identify mechanical thresholds for spinal cord injury in the rat. The Impactor model is the gold standard in vivo technique for studying SCI and has been extremely well characterized in terms of the biological, physiological, and functional deficits that follow injury; yet, the biomechanics that underlay the model – which by definition are what cause the injury – have received only cursory investigation. We will examine biomechanical injury to the microvasculature of the spinal cord, which leads to a number of secondary pathologies that impact other cell populations in the spinal cord. The specific aims of this proposal are unchanged and are:

1) Identify mechanical stress and strain in the spinal cord as a function of time during SCI
2) Correlate stress and strain to the extent and severity of microvascular injury.

2. Project Successes

In whole, the project has been a great success. We successfully completed an experimental study documenting immediate breakdown of the blood-spinal cord barrier following weight drop trauma in rats. Three markers of distinct sizes were examined – molecule-sized (hydrazide), protein-sized (albumin), and cell-sized (red blood cells). The volume of injury was correlated to mechanical parameters recorded during the experiment, such as compression depth and rate. The work was published in the Journal of Neurotrauma (see included article), and the study also provided spatial 'maps' of injury that documented where the 3 species crossed the mechanically damaged blood-spinal cord barrier (Figure 1). This was critical for comparisons to our finite element model of spinal cord mechanics.

We then simulated the weight drop experiment with computational techniques. The 3D geometry of the spinal cord was recreated from magnetic resonance images, and the geometry of the spinal column was recreated from microCT images. The spinal cord include the gray and white matter as distinct entities. We added the cerebrospinal fluid layer and a dura mater based on the outer geometry of the spinal cord. We then meshed the spinal cord, CSF, and dura into small volumes, termed finite elements, and specified mechanical properties, boundary conditions, and loading conditions for each element. The spinal cord was given properties from the literature. The CSF was assigned fluid-like properties. However, there was no available data on rat dura mater (spinal or cranial). As such, we completed a series of mechanical tests on rat spinal and cranial dura mater to characterize the elastic and viscoelastic properties. We found that both tissues could be modeled as hyperelastic-linearly viscoelastic, and that collagen fibers in the spinal dura demonstrated significant axial alignment, whereas no preferential alignment could be found in cranial dura. This work has been submitted the Journal of Neurotrauma, and the editors asked for 'Minor Revisions' (see below). We expect to re-submit the manuscript within 2 weeks and anticipate a positive response based on the first reviews.

With material properties in hand, we simulated the weight drop experiments. A rigid impactor was added (with the exact size and weight of those used in experiments) and placed on top of the exposed dura of the model. The spinal column was fixed in space so that it could not move. The impactor was then prescribed a velocity and acceleration that matched the average from experiments. The properties of the spinal cord were adjusted until the trajectory of the impactor in the simulations fell within the 95% confidence intervals of the experimental data. The simulations demonstrated that stress and strain is well correlated to the patterns of primary injury, and that adding anisotropy to the model by changing the properties of gray or white matter, while not changing rod trajectory, did significantly alter the tissue stress and strain. The stress and strain patterns tended to better match experiments when white matter was stiffer than gray matter. Simulations were also run where the impactor was placed off-center by a fraction of a millimeter. Although the impactor trajectory was very similar to that from a centered impact, the location of stress and strain 'hot spots' shifted, and may explain asymmetric injury patterns and or unusual functional deficits where an animal favors one leg over another following weight drop injury. This work is also under review at the Journal of Neurotrauma (see below).

Finally, the simulations were quantitatively compared to the experiments using a novel statistical procedure. Three longitudinal slices from the mesh were extracted and superposed on the injury maps from the weight drop experiments. Each element was graded as either injured or uninjured, depending on whether it overlapped with an injured area or not (Figure 1). Then the injury status was plotted against the maximum value of different mechanical parameters gleaned from the simulations, and a logistic regression was performed. This was completed for each experiment at 12.5mm and 25mm drop heights, for each of the extravasated markers, and the specificity and sensitivity of each predicted injury threshold identified (Figure 2). The compiled data revealed that: A) maximum strain is the best predictor of gray matter injury; B) von Mises stress was the best predictor of white matter injury, but the results were not as statistically consistent as for gray matter (Figure 3). This suggested that an inhomogeneous model, where gray and white matter are prescribed different material properties, as described above, would likely yield better results. We are now completing an analysis of the inhomogeneous models. We expect to submit a manuscript on these results by the end of the calendar year.

3. Project challenges

An original goal of the project was to identify different properties for rat spinal cord gray and white matter using MRI by compressing the spinal cord with varying force in the magnet, recording the subsequent deformation in images, and the identifying what properties would allow the gray and white matter to deform as such using an 'inverse engineering' computational approach. This proved to be too challenging both logistically (MR experiments were performed at the University of Pennsylvania), financially (MR time is expensive), and technically (a non-magnetic had to be built to apply controlled force to the exposed rat spinal cord in the magnet).

The computational model was difficult to complete as well. A number of compromises were made – we were unable to model the CSF as a true fluid, and instead modeled it as a solid with fluid like behavior by prescribing a low shear modulus and high bulk modulus; we were unable to simulate experiments from a 50mm drop height because the deformation was too severe; and with our finite element code, were are unable to introduce anisotropic material properties to the spinal cord, which would allow the white matter to be stronger in the direction of axons vs. perpendicular to axons. The model also required extensive computational power, and the research would not have been completed without the generous support of Victor Barocas and the University of Minnesota Supercomputing Institute.

4. Implications for future research and/or clinical treatment

This research has great implications for the prevention of spinal cord injury. The work has predicted an injury threshold for the gray matter of ~8% strain for hydrazide, 11.5% strain for albumin, and 14% strain for red blood cells. These strains are at the tissue level, and should hold regardless of the nature of mechanical trauma. This can best be validated by inducing injury with another model (eg electromagnetic impactor). These strains can then be incorporated into full models of spinal impact (vs. direct insult to the spinal cord/dura) to begin to predict what conditions will cause injury to the spinal cord, not just the spine. The conditions then server as benchmarks to avoid when implementing safety standards and designing new safety equipment. The work also identifies appropriate loading conditions to study spinal cord injury in vitro, where stresses and strains are placed directly on cells or slices of tissue. Future research should focus on better identification of material properties for gray and white matter in the rat, and age-specific changes in these properties. In this work, we specifically characterized injury to the blood-spinal cord barrier; however, the methodologies can be repeated for injury to other entities, such as axons. Additionally, the research paradigm can be repeated in a mouse model, which would allow for the investigation of the influence of transgenic mutations on primary spinal cord injury.

5. Plans to continue research

We have submitted a proposal for an Individual Research Grant to the NJCSCR to utilize these methods to characterize axonal injury in the rat. Additionally, we are submitting a proposal in February to the National Center for Injury Prevention and Control at the CDC, which combines this work with separate research in

our lab aimed at understanding the biomechanics of axonal injury as the axon level (vs. tissue level herein). Finally, we hope to submit a proposal for simulating injury in the mouse in the Spring.

6. Peer-reviewed publications:

Maikos, J.T. and *Shreiber, D.I.*. Immediate damage to the blood-spinal cord barrier due to mechanical trauma. J. Neurotrauma, 2007. 24(3): 492-507.
Submitted:
Maikos, J.T., Elias, R.AI., and *Shreiber, D.I.*, Mechanical properties of rat brain and spinal cord dura mater in uniaxial tension. J. Neurotrauma..

Maikos, J.T. Qian, Z., Metaxas, D., and Shreiber, D.I. Finite element modeling of spinal cord injury. J. Biomechanical Engineering.

In preparation:
Maikos, J.T. and Shreiber, D.I. In vivo thresholds for spinal cord injury. Journal of Biomechanics. Expected December 2007.

Peer-reviewed conference proceedings:

Maikos, J.T., Qian, Z., Metaxas, D., and *Shreiber, D.I.* In vivo thresholds for spinal cord injury. 2007 ASME Summer Bioengineering Conference, Keystone, CO.*Honorable Mention, PhD Student Platform Presentation Competition

Maikos, J.T., Señeres, A.W., Monteiro, G., Qian, Z., Metaxas, D., and *Shreiber, D.I.* In vivo tissue-level thresholds for spinal cord injury. 2005 ASME Summer Bioengineering Conference, Vail, CO, June, 2005. *Honorable Mention, PhD Student Platform Presentation Competition

Abstracts:
Elias, R.AI., Maikos, J.T., and *Shreiber, D.I.* Mechancial properties of the developing chick spinal cord. 2007 ASME Summer Bioengineering Conference, Keystone, CO.

Maikos, J.T., and *Shreiber, D.I.* Developing a Finite Element Simulation of In Vivo Models of Spinal Cord Trauma. 2006 Biomedical Engineering Society Meeting, Chicago, IL.

Maikos, J.T., Patel, D., Young, W., and *Shreiber, D.I.* Characterization of immediate blood-spinal cord barrier injury due to mechanical trauma. 2005 Biomedical Engineering Society, Baltimore, MD. *Award for outstanding innovation in biomedical research (~5% of posters were given the award)

Maikos, J.T., Patel, D., Young, W., and *Shreiber, D.I.* Characterization of immediate blood-spinal cord barrier injury due to mechanical trauma. 2004 Society for Neurotruama Symposium, November, 2004, San Diego, CA.

Maikos, J.T., Patel, D., Young, W., and *Shreiber, D.I.* Characterization of immediate blood-spinal cord barrier injury due to mechanical trauma. 2004 BMES Annual Meeting, October, 2004. Philadelphia, PA.

Figures: We have not included separate figures from the manuscripts published or submitted, which are appended.

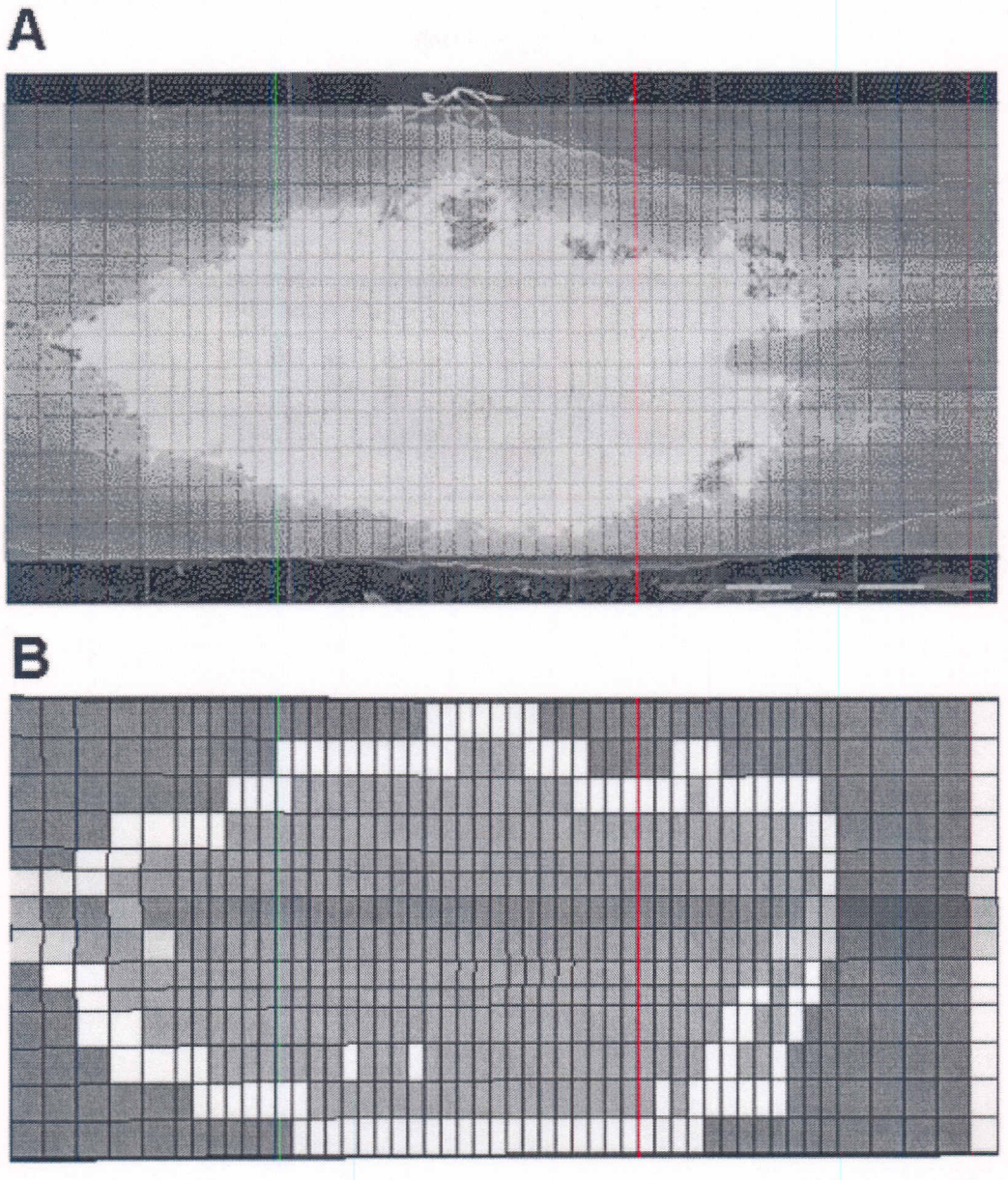

Figure 1: A section of the finite element mesh (grid) is extracted from the model and superposed over a slice of spinal cord sowing injury. Elements are marked as injured (if it overlaps with an injured area - red) or uninjured (if not - blue).

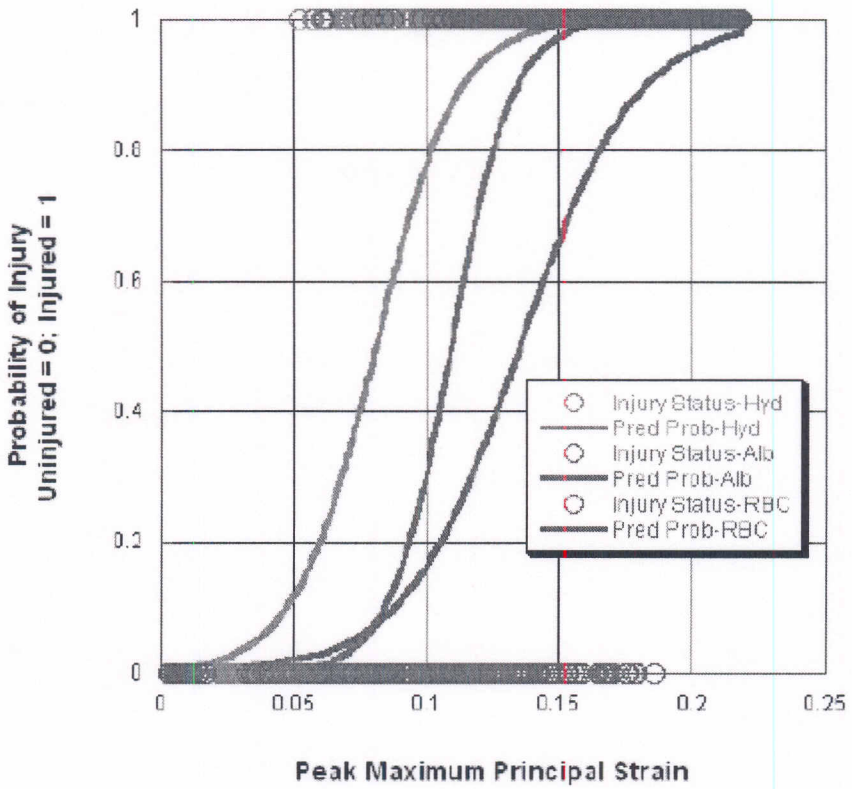

Figure 2: The injury status (1 = injured, 0 = uninjured) is plotted against a peak mechanical measure, such as stress or strain (shown). A logistic regression is performed. The steeper the 'S' shaped curve, the better the regression. These regressions were performed for the 3 markers of injury for each experiment at 2 drop heights, and the optimal threshold identified based on the specificty and sensitivity of the injury prediction.

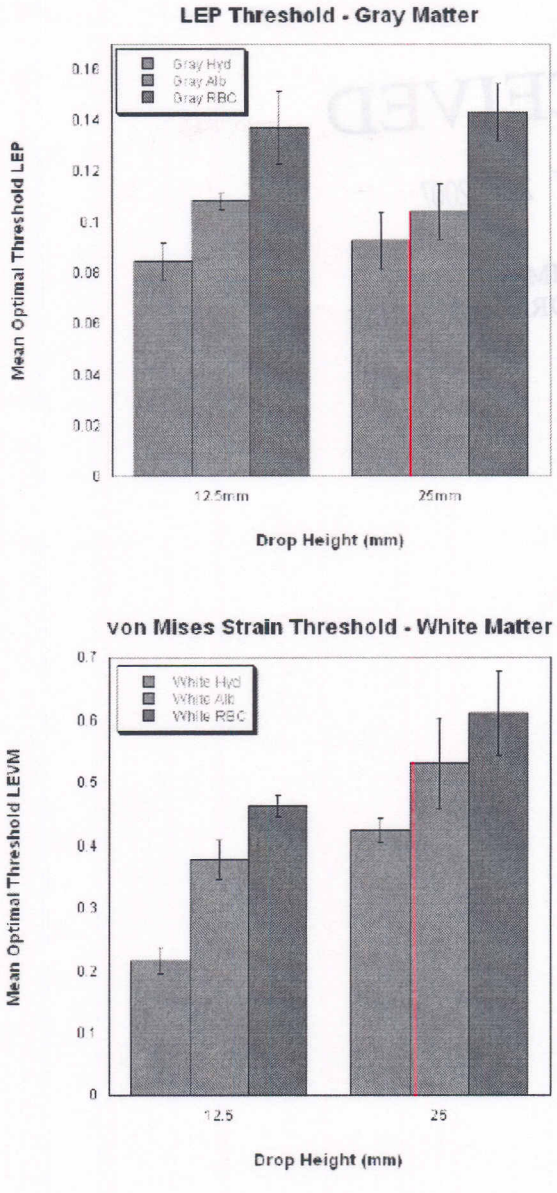

Figure 3: The injury thresholds for gray matter were consistent between the 12.5mm and 25mm experiments. More strain was required to cause injury severe enough for red blood cells to cross the blood-spinal cord barrier (ie hemorrhage) than proteins , which required more strain than small molecules. Individual predictions of thresholds for injury to white matter were very strong based on their regression coefficients, but collectively were not consistent between the two drop heights. The results suggest that an inhomogeneous and perhaps anisotropic model is required to predict injury to white matter.